Data Evaluation Report on the Acute Dietary Toxicity of Technical Thidiazuron to Northern Bobwhite Quail (*Colinus virginianus)*

PMRA Submission Number EPA MRID Number 46203502

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D294536
OECD Data Point	
EPA MRID	46203502
EPA Guideline	§71-2a

Test material: Technical Thidiazuron **Purity:** 99.5%
Common name: Thidiazuron
Chemical name: IUPAC: *N*-Phenyl-*N'*-1,2,3-thiadiazol-5-ylurea
CAS name: Not reported
CAS No.: 51707-55-2
Synonyms: None reported

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Date: 4/8/04

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Primary Reviewer:
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Date: 11/16/04

Secondary Reviewer(s):
{EPA/OECD/PMRA}

Date:

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Company Code:
Active Code:
EPA PC Code: 120301

Date Evaluation Completed:

CITATION: Bowers, L.M. 2003. Technical Thidiazuron: A Subacute Dietary LC_{50} With Northern Bobwhite. Unpublished study performed by Bayer Corporation, Stilwell, KS. Laboratory Study No. TN721701. Study submitted by Bayer CropScience, Research Triangle Park, NC. Study initiated January 13, 2003 and completed March 12, 2003.

EXECUTIVE SUMMARY:

The acute dietary toxicity of Thidiazuron Technical to 13-day-old Northern Bobwhite quail (*Colinus virginianus*) was assessed over 8 days (5 days with treated feed and 3 day recovery period). Thidiazuron Technical was administered to the birds in the diet at nominal concentrations of 0 (negative control) and 5000 ppm (limit concentration). Mean-measured concentrations were <500 (LOQ, controls) and 5215 ppm a.i.

No treatment related effects on mortality, clinical signs of toxicity, body weight, or feed consumption were observed. Necropsy revealed no gross lesions. The 8-day LC_{50} was >5215 ppm a.i., which categorizes Thidiazuron Technical as practically non-toxic to Northern Bobwhite quail on an acute dietary basis.

This toxicity study is deemed scientifically sound, satisfies the guideline requirement for an avian dietary study with the Northern Bobwhite Quail (§71-2a), and is classified as CORE.

Results Synopsis

Test Organism Size/Age: 13-day old chicks, 44.2-50.5 g

LC_{50}: >5215 ppm a.i.
NOEC: 5215 ppm a.i.
LOEC: >5215 ppm a.i.
Endpoints affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The protocol followed procedures of the U.S. EPA FIFRA Guideline §71-2 (1982), and the ASTM method "Standard Practice for Conducting Subacute Dietary LC_{50} Tests with Avian Species" E857-87 (1993). The following deviations from §71-2 guidelines were noted:

1. The brooder size (36 x 30 x 10 cm) was significantly less than required (about 35 x 100 x 24 cm).

This deviation did not have a significant effect on the results of the study, and was considered minor.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study meets the requirements of 40 CFR Part 160 with the exception of the feed contaminant analysis.

A. MATERIALS:

1. Test Material Technical Thidiazuron

Description: Light yellow powder

Lot No./Batch No.: 107623-03

Purity: 99.5%

Stability of Compound Under Test Conditions: Stability was assessed in treated feed after 1 day of brooder storage and after 15 days of frozen storage (p. 12). The brooder recovery was

106% of the initial concentration, and the freezer recovery was 101% of the initial concentration (Appendix 2, p. 31).

Storage conditions of test chemical: Ambient laboratory temperature

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

2. **Test organism:**

Species: Northern Bobwhite Quails (*Colinus virginianus*)

Age at study initiation: 13 days old

Weight at study initiation: 44.2-50.5 g (Appendix 3, p. 35-36); group means of 46.2-47.2 g

Source: Barrett's Quail Farm, Houston, TX

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: None conducted.

b) Definitive Study:

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	10 days, followed by a 3-day pre-exposure period	Eggs were obtained from Barrett's Quail Farm on December 5, 2002, and were incubated and hatched at the laboratory.
Conditions (same as test or not):	Same as test	
Feeding:	Teklad Bayer Starter ration and local tap water were provided *ad libitum*.	
Health (any mortality observed):	All birds appeared healthy. No mortality during 3 days prior to test initiation.	
Pen size and construction materials	Galvanized steel brooders that measured 36 x 30 x 10 cm	*EPA requires: about 35 x 100 x 24 cm*

Parameter	Details	Remarks *Criteria*
Test duration	5 days with treated feed, and 3 days with"clean" feed.	*EPA requires: 5 days with treated feed and at least 3 days observation with "clean" feed.*
Test concentrations nominal: measured:	0 (negative control) and 5000 ppm <500 (<LOQ; control) and 5215 ppm a.i.	Measured concentrations are reported in Table 1, p. 16. *Four minimum, 5 or 6 strongly recommended, in a geometric scale, unless LC_{50} > 5000 ppm.*
Solvent/vehicle, if used type: amount:	N/A	*EPA requires: Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic. Solvent not more than 2%.*
Diet preparation and feeding	The appropriate amount of test chemical was sieved, combined with a portion of basal diet, and blended for at least 5 minutes with a Kitchen Aid mixer (p. 11). This premix was then quantitatively transferred to a Hobart mixer which contained approximately half of the basal diet, and mixed for approximately 10 minutes. The remaining half of basal diet was added, and mixed for an additional 10 minutes. Feed was prepared on January 6, 2003, and stored frozen until needed. Fresh diets were offered each day.	*EPA requires: Control group tested with diet containing the maximum amount of vehicle used in treated diets?*
Feed withholding period	None	
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	Yes	
Number of birds per replicate/group for negative control: for solvent control: for treated:	 10 N/A 10	*EPA requires: 10 (strongly recommended)*

Parameter	Details	Remarks *Criteria*
Number of replicates/group (if used) for negative control: for solvent control: for treated:	 2 N/A 1	
Test conditions temperature: relative humidity(%): photoperiod:	 Approximately 22°C for room temperature and 30-38°C for brooder temperatures. Approximately 55% 14 hours light/10 hours dark	 ***Brooder temperature:*** *about 35°C (95°F)* ***Room temperature:*** *22-27°C (71-81°F)* ***Relative humidity:*** *30-80%* ***Photoperiod:*** *Minimum of 14 h of light.*
Reference chemical, if used	None used.	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured (mortality/body weight/ mean feed consumption/ others)	- Mortality - Clinical signs of toxicity - Mean feed consumption (g/bird/day) - Mean body weight - Necropsy	

Indicate the stability and homogeneity of test chemical in the diet	Stability: Verified. Stability was assessed in treated feed after 1 day of brooder storage and after 15 days of frozen storage (p. 12). The brooder recovery was 106% of the initial concentration, and the freezer recovery was 101% of the initial concentration (Appendix 2, p. 31). Homogeneity: Verified. Homogeneity was assessed from nine locations of the prepared treated feed (p. 12). The coefficient of variation was 5% (Table 2 of Appendix 2, p. 32).	Treated feed was prepared on January 6, 2003 and stored frozen (p. 12). On a daily basis during the 5-day treatment period, frozen feed was removed and provided to the quail. On January 21, 2003, samples were collected for concentration analysis from the brooders following completion of the feed consumption measurements (representing 1 day brooder stability), and additional samples were collected for concentration analysis from the treated feed that had been maintained in the freezer (representing 15 day freezer stability).
Indicate if the test material was regurgitated	Regurgitation was not reported.	
Treatments on which necropsies were performed	All surviving birds were subject to a gross pathological examinations.	
Observation intervals	Mortality and signs of toxicity were recorded approximately 2, 3, and 5 hours on Day 0, and twice daily thereafter, except on weekends and Day 8, when only one observations was made. Feed consumption was determined daily. Body weight was determined on Days -3, 0, 5, and 8.	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortality occurred during the study (p. 13). The LC_{50} was >5215 ppm a.i.

Table 3: Effect of Technical Thidiazuron on mortality of Northern Bobwhite Quails.

Treatment, ppm a.i. mean-measured (and nominal)		No. of birds per treatment	Cumulative mortality								
			Days								
			0	1	2	3	4	5	6	7	8
Negative controls		20	0	0	0	0	0	0	0	0	0
5215 (5000)		10	0	0	0	0	0	0	0	0	0
NOEC		5215 ppm a.i.									
LC_{50}		>5215 ppm a.i.									
Reference chemical	mortality	N/A									
	LC_{50}	N/A									
	NOEC	N/A									

B. SUB-LETHAL TOXICITY ENDPOINTS:

No clinical signs of toxicity were observed during the study (p. 13).

Body weight on Day 5 and growth (body weight changes) between Days 0 and 5 were statistically reduced for the treatment group compared to the controls (pp. 13-14, and Table 5, p. 20). On Day 5, body weights averaged 72.2 and 72.7 g for the two control groups, and 68.0 g for the treatment group. Growth during the exposure period averaged 25.0 and 25.8 g for the two control groups, and 21.8 g for the treatment group. Body weights on Day 8 and growth between Days 5 and 8 were comparable to control values. The study author reported that since chick growth and body weight exhibited recovery during the study, the NOEC for body weight and growth was 5215 ppm a.i.

Since no replicate data existed for this endpoint (due to the study design), statistical analysis could not be performed on feed consumption (p. 14). The treated birds had slightly lower food consumption during the exposure (10% inhibition) and recovery (2% inhibition) periods. The study author reported that these differences did not appear to be significant, and concluded that the NOEC for feed consumption was 5215 ppm a.i.

Table 4: Sub-lethal effects of Thidiazuron Technical on Northern Bobwhite Quails.

Treatment, ppm a.i. mean-measured (and nominal)		Observation						
		Mean body weight (g)			Growth (g)		Food consumption (g/bird/day)[1]	
		Day			Exposure Period	Recovery Period	Day	
		0	5	8			0-4	5-7
Negative control group 1		47.2	72.2	87.1	25.0	14.9	9.0	10.5
Negative control group 2		46.9	72.7	86.7	25.8	14.0	9.0	10.4
5215 (5000)		46.2	68.0*	83.4	21.8*	15.4	8.1	10.3
NOEC		5215 ppm a.i.			5215 ppm a.i.		5215 ppm a.i.	
EC_{50}		Not determined			Not determined		Not determined	
Reference chemical	NOEC	N/A						
	EC_{50}	N/A						

[1] The feed consumption data was not statistically analyzed because there was no replicate data.
* Statistically significant from the control (Kruskal-Wallis ANOVA; p>0.05)

C. POST-MORTEM EXAMINATION:

No gross lesions were observed at necropsy (p. 13, and Table 4, p. 19).

D. REPORTED STATISTICS:

The LC_{50} could not be calculated because mortality did not exceed 50% at any test level. Since there was only one treatment group, the Kruskall-Wallis ANOVA by ranks and the Dunn's multiple comparison test was used to detect significant differences among the treated and control groups (p = 0.05). The mean-measured concentration was used in the estimations. Feed consumption data were not analyzed statistically, as there were no replicate data.

LC_{50}: >5215 ppm a.i.
NOEC: 5215 ppm a.i.
LOEC: >5215 ppm a.i.
Endpoint(s) Affected: None

E. VERIFICATION OF STATISTICAL RESULTS:

The LC_{50} could be determined visually, as mortality did not occur in this study. Body weight data were not statistically analyzed. The reviewer could visually determine that growth was reduced during the exposure period, but there was no difference between control and treatment during the recovery period. Furthermore, food consumption in the treated group was lower than control during days 0-2 of the exposure period, while no reduction was detected during the remainder of the study. This shows that the reduction in body weight during the exposure period may have been related to a food aversion during the initial days of exposure to treated feed.

LC_{50}: >5215 ppm a.i.
NOEC: 5215 ppm a.i.
LOEC: >5215 ppm a.i.
Endpoint(s) Affected: None

F. STUDY DEFICIENCIES:

No notable deficiencies were observed in this study. As a result, this study fulfills guideline requirements for an avian dietary study using the Northern Bobwhite Quail (§71-2), and is classified as CORE.

G. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study author's. There was no mortality in this study; the 8-day LC_{50} was >5215 ppm a.i., which categorizes Thidiazuron Technical as practically non-toxic to Northern Bobwhite quail on an acute dietary basis. Body weights were reduced during the exposure period, which was likely related to food aversion during the initial days of exposure to treated feed. There were no differences between the treatment and control groups in food consumption or body weight during the recovery period.

All birds were de-beaked (clipped the tip of the upper beak) on the day of hatch to reduce intraspecific aggression, or cannibalism (p. 10).

The analytical report provided was in conjunction with the Northern Bobwhite Quail LC50 toxicity study (Bayer Laboratory No. TN721701; p. 27).

In a method validation experiment, control feed was fortified with Technical Thidiazuron at 500 and 4998 ppm. The average recovery at the 500 ppm level was 101 ± 1%, and the average recovery at the 4998 ppm level was 104 ± 2% (Appendix 2, p. 30).

A laboratory spike of 4998 ppm was prepared and analyzed with the feed of each sampling interval. The concurrent recoveries averaged 104% of nominal (Appendix 2, p. 30).

H. CONCLUSIONS:

This toxicity study is scientifically sound, fulfills the guideline requirements for an avian dietary study using the Northern Bobwhite Quail (§71-2a), and is classified as CORE. Based on the results of this study, Technical Thidiazuron is categorized as practically non-toxic to Northern Bobwhite quail on an acute dietary basis.

LC_{50}: >5215 ppm a.i.
NOEC: 5215 ppm a.i.
LOEC: >5215 ppm a.i.
Endpoints affected: None

III. REFERENCES:

American Society of Testing and Materials (ASTM). 1993. Standard Practice for Conducting Subacute Dietary Tests with Avian Species. ASTM Standard E857-87.

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